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Research Article

TRACER EXPERIMENTS WITH I^{131} LABELED HUMAN SERUM ALBUMIN: DISTRIBUTION AND DEGRADATION STUDIES

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The distribution and metabolism of serum proteins in human subjects have been investigated recently by means of tracers labeled *in vitro* with I^{131} or biosynthesized with S^{35} or N^{15}. A study by London (1) indicated a half time of about 20 days for N^{15} labeled serum albumin turnover in a normal man and woman. In two normal subjects an average half time value of 17.1 days for serum albumin was obtained by Volwiler, Fremont-Smith, and MacMartin (2) following the oral administration of S^{35} tagged dl-cystine. Sterling (3), however, utilizing the disappearance rate of injected I^{131} tagged human serum albumin, reported a mean half time of 10.5 ± 1.5 days in 21 normal medical students, the longest being 13.4 days. Similarly, marked differences in the average half times of gamma globulin as determined by I^{131} labeling and S^{35} biosynthesis have been reported. Values of 7.0 to 8.8 days (4) and 7.8 days (5) have been found by the former method as contrasted with 27.0 days by the latter (2).

These discrepancies merit further investigation into the validity of the methods employed, especially in view of the increasing application of I^{131} tagged proteins to the study of turnover rates in health and disease. In the present study the fate of intravenously administered I^{131} labeled human serum albumin has been followed over periods of time considerably longer than have heretofore been reported. The results emphasize the difficulties in interpretation of data and highlight the shortcomings of this method of analysis. They also aid in resolving the discrepancies noted above.

METHODS

Iodination of albumin. Lots of albumin iodinated by three different techniques were utilized. Two methods were employed in the laboratory and several batches prepared by each were studied.[1] The final products contained an average of 1 to 2 atoms of iodine per molecule of protein except in one case. Method A was identical with that reported by Pressman and Eisen (6). When relatively large amounts of I^{131} solution[2] were used, only 8 to 10 per cent of the radioactive iodine became protein bound, probably owing to the reducing effect on elemental iodine of the acid sodium sulfite accompanying the I^{131}. In order to increase the efficiency of radioactive labeling and to decrease radiation exposure during preparation, method B (7) was used. In this case, the elemental iodine was similarly formed from a mixture of stable and radioactive iodide by nitrous acid but it was protected from reduction to iodide by its removal from the mixture into a chloroform phase. Since the iodine-protein combination occurs rapidly the chloroform-iodine solution was added slowly to the continually stirred solution of albumin in order to obtain optimal random iodination. By this procedure, 25 to 35 per cent of the I^{131} became protein bound. As is indicated below, there has been no reason to suspect an adverse effect of the chloroform on the albumin, the lots prepared by this method proving the most satisfactory of all those studied. In many experiments, labeled albumin prepared by Abbott Laboratories and specified to contain 1 to 2 I atoms per molecule protein was used. Although, before shipment, this material had been passed through resin columns designed to absorb residual iodide and iodate, usually about 3 to 5 per cent and occasionally as much as 8 per cent was found to pass a Visking cellulose membrane upon receipt in the laboratory. Therefore, all lots of iodinated albumin utilized were dialyzed in the cold against frequently changed normal saline solutions for about 48 hours. Lots prepared in this laboratory were passed through Seitz filters and tested for sterility by culture before use. Cold 10 per cent trichloracetic acid precipitated 98 per cent or more of the radioactivity in the final products.

Ultracentrifugal and electrophoretic analysis of iodinated proteins. Sample lots of labeled albumin were subjected to ultracentrifugal[3] and electrophoretic analysis. The electrophoretic patterns[4] showed a single peaked symmetrical curve identical to that obtained with "native"

[1] We are indebted to the American National Red Cross for supplies of "normal" human serum albumin manufactured and packed by the Armour Laboratories, Ft. Worth, Texas.

[2] Obtained from Oak Ridge National Laboratory.

[3] Thanks are due to Dr. Kurt G. Stern, Brooklyn Polytechnic Institute, for the ultracentrifuge analyses.

[4] 0.5 per cent solution, phosphate buffer, pH 7.4.

albumin. Mixtures composed of equal parts of iodinated and native albumin also showed a single peak. Similarly, ultracentrifugal analysis usually failed to detect inhomogeneity or differences between labeled and native albumin, even when the biological behaviour of the labeled albumin was such as to suggest altered protein, at least in part.

Assay of radioactivity. Several counting systems were simultaneously employed, and each was standardized with respect to the others. *In vivo* assays were performed with a bismuth walled Geiger counter replaced daily in an identical position with respect to that part of the body being assayed. At 5 and 9 inches respectively, this counter had a sensitivity of approximately 250 and 100 counts per minute per μc of I^{131}, depending on the *in vivo* source of radioactivity, above a background of 60 counts per minute. Because of a wide window in the counter shield exposing the entire sensitive region of the tube (five inches $\times$ one inch), a fair sized volume of even such large organs as the liver could be "seen" by the counter at these distances. The rates of disappearance of radioactivity from sites of injection of tagged albumin were determined by methods previously described (8, 9). In some cases a scintillation counter was used.

Radioactivity of stool, urine and ascitic fluid specimens was assayed in 100 ml. samples in Marinelli beakers placed around bismuth walled or tetramethyl lead filled silver walled glass Geiger counters with sensitivities of 5200 counts per minute per μc I^{131} above a background of 28 counts per minute and of 479 counts per minute per μc I^{131} above a background of 16 counts per minute, respectively. Plasma and ascitic fluid were assayed in 5 ml. samples in specially modified Marinelli-type beakers placed around the bismuth tube. A sensitivity of 10,500 counts per minute per μc I^{131} was obtained with this arrangement. Duplicate 0.5 ml. plasma samples were dried in circular planchets of 2 cm. diameter and assayed in a flow gas counter. The sensitivity of the arrangement was 1.02 to 1.10×10^6 counts per minute per μc I^{131} depending on the density of the plasma, above a background of 20 counts per minute. The sensitivity could be precisely determined for each subject's plasma by the addition of a known amount of I^{131}. Five ml. samples were also occasionally assayed in a well-type sodium iodide crystal scintillation counter with a sensitivity of 0.96×10^6 counts per minute per μc I^{131} for this volume, above a background of 432 counts per minute. Suitable dilutions of the administered doses were assayed in all counters and kept throughout the study for frequent intercomparisons and for verification of stability of the various counting arrangements. In a number of experiments all plasma samples were assayed in both the flow gas and bismuth counters and some samples assayed in the scintillation counter as well. The precision obtained in replicate samples assayed in different counters indicated that counting techniques were not a source of significant variation. A sufficient number of counts was recorded so as to reduce the statistical error of counting to below 3 per cent in the least active plasma specimens.

Subjects studied. These were male patients of the Veterans Administration Hospital, Bronx, New York. One hundred to 150 μc I^{131} tagged albumin (1 to 20 mgm.) from different lots were administered intravenously from weighed syringes to groups composed of one to seven subjects. The larger groups included cirrhotic and noncirrhotic patients. There were 53 separate studies in 46 subjects. Inhibition of thyroid uptake was effected by the administration of 10 drops of Lugol's solution by mouth three times a day throughout the study. 24 hour urine collections were obtained daily except in an occasional instance when a pooled weekend urine was collected. Heparinized blood specimens were drawn without tourniquet stasis at frequent intervals for periods up to 43 days following administration of the labeled albumin. While in many early experiments blood samples were drawn from the recumbent subject after rest, this practice was subsequently discontinued as an unnecessary refinement in long term studies. Serum albumin concentrations were determined at 7 to 10 day intervals from micro Kjeldahl nitrogen analysis after Howe sodium sulfate fractionation. Ascitic fluid albumin concentrations were similarly determined. In all but one case (J. B., No. 10), these concentrations remained constant within the limits of error of the method. Plasma volumes were determined from the 15 minute dilution factors according to methods previously described (10).

Localization of radioactivity in plasma and ascitic fluid. The estimation of turnover rates from the analysis of plasma radioactivity requires that the radioactivity remain bound to the undegraded albumin. This condition has been examined by the dialysis of plasma, the precipitation of plasma proteins and the assay of radioactivity in electrophoretically fractionated plasma at various intervals following the administration of tagged albumin. In all experiments less than 4 per cent of the plasma radioactivity passed a dialysis membrane, more than 94 per cent of the radioactivity was precipitable with cold 10 per cent trichloracetic acid and all of the radioactivity was accounted for in the albumin fraction separated by electrophoresis. It should be appreciated, however, that separation by this latter method is crude and that this fraction undoubtedly also contained some alpha-globulin. Approximately 98 per cent of ascitic fluid radioactivity was precipitable with cold 10 per cent trichloracetic acid.

Definition of terms.

Rate—Apparent instantaneous fractional rate; this may be an average of several indistinguishable rates.

Compartment—An apparent space of distribution not necessarily confined to a single anatomically bounded region except in the case of plasma.

Iodo-Albumin Component—A moiety of iodo-albumin composed of a single biologically homogeneous substance or of several different substances characterized by closely similar degradation rates.

Iodo-Albumin Degradation—The separation of radioactivity from the I^{131} labeled albumin which does not necessarily imply total or partial hydrolysis of the protein.

ANALYSIS OF DATA AND RESULTS

I. *Construction and analysis of curves*

The method of plotting observed and derived data and the significance of the curves obtained are best followed from the study of a representative experiment (Figure 1). In each case, the following curves were drawn. All values were corrected for radioactive decay and plotted as a function of time on semilogarithmic paper.

A) *"Plasma concentration curve."* Counts per minute per unit volume plasma. After distribution in the body's exchangeable albumin pool is essentially complete and in the absence of proteinuria or other external losses, the slope of this curve defines the rate of tagged albumin degradation. It has been inferred (3) that the rate of albumin synthesis is equal to this rate if the concentration and space of distribution of serum albumin remain constant. In one case (J. B., No. 10), be-

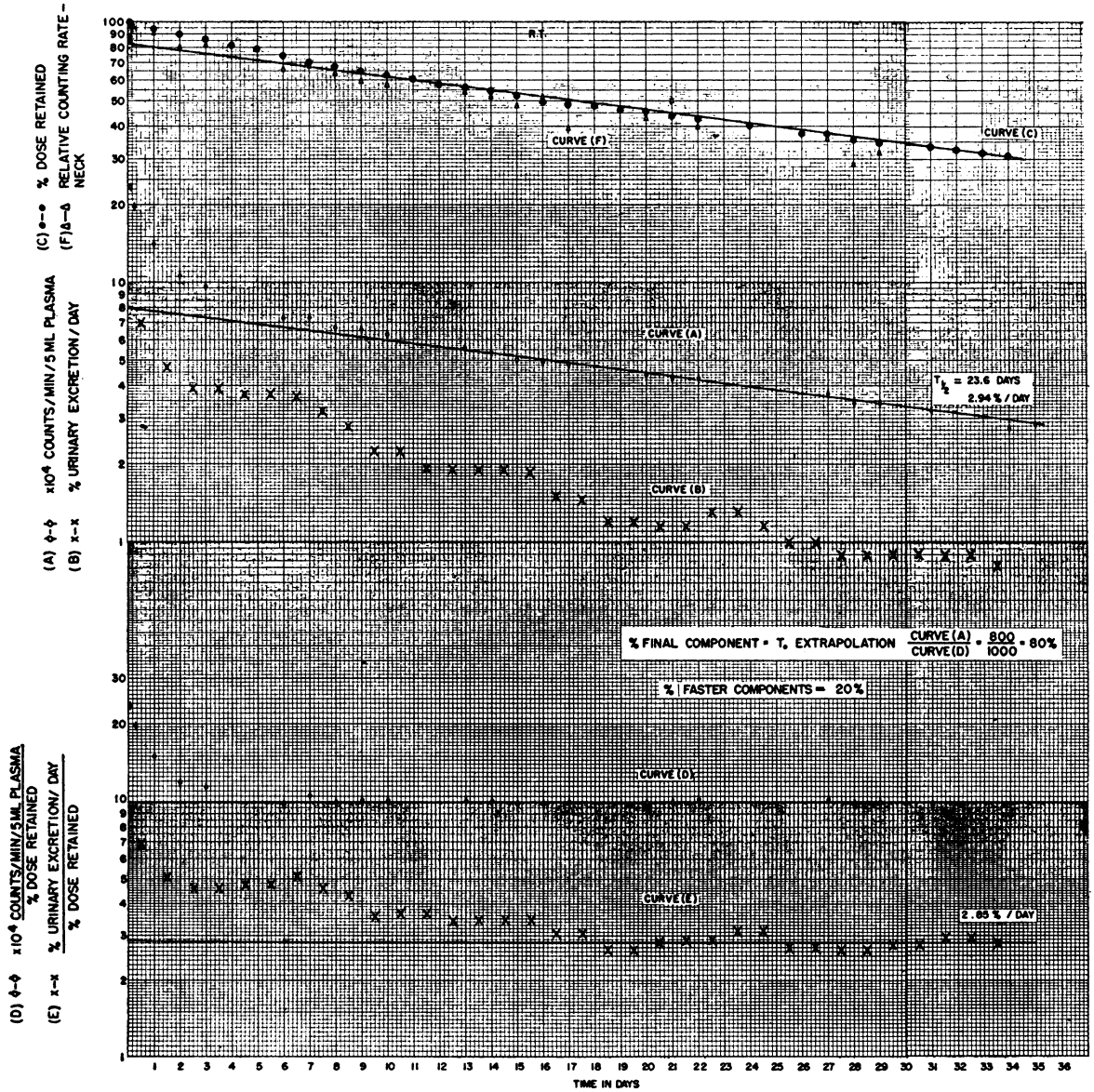

FIG. 1. TYPICAL SET OF CURVES DERIVED FROM DATA IN SUBJECT R. T. (NO. 15) (SEE TEXT FOR INTERPRETATION)

In this and subsequent figures some of the urine points represent the means of two day collections. The first point represents the actual excretion divided by the fraction of a 24 hour period over which it was collected.

cause of a wide variation in plasma albumin concentrations (± 15 per cent of mean), specific activities (counts per minute per gram albumin) rather than radioactivities were plotted.

B) *"Renal excretion curve."* The per cent of the administered dose excreted daily in the urine is plotted at the midpoint of the day. In one case (S. H., No. 43) significant proteinuria was present and separate assay of the protein bound and protein free radioactivity was made.

C) *"Per cent retained."* The per cent of the dose retained in the body at any time is considered to be equal to 100 per cent minus the cumulative per cent urine excretion on the assumption that all the radioactivity lost from the body is recovered in the urine collections. The validity of this assumption is confirmed or negated, in any individual case, by the characteristics of the forms of curves D) and E) described below. Approximately 2 to 3 per cent of the radioactivity was withdrawn during blood sampling during the course of the study. These losses were corrected for in calculation of the per cent retained. In three subjects occasional 24 hour stool analyses showed that fecal excretion amounted to only .2 per cent to 2.5 per cent of that excreted in the urine. Fecal excretion was therefore disregarded.

D) *"Distribution curve."* Counts per minute per unit volume plasma/per cent retained. On the assumption that all radioactivity released from the degraded protein is quantitatively accounted for by renal excretion, this curve reflects the changes in plasma concentration due solely to distribution of the tagged albumin in the exchangeable albumin pool. If the assumption is valid, this curve should approach an asymptote parallel to the time axis as mixing becomes more complete. It should have a negative slope if recovery in the urine does not account for all the degraded protein radioactivity. The reciprocal of this curve would indicate the time course of the apparent space of distribution.

E) *Daily renal excretion (per cent of dose)/per cent retained.* If the assumption made in C) is valid, then the absolute level of this curve at any time expresses the per cent of the tagged protein within the body which is being degraded daily at that time.

F) *In vivo counting rate over neck and/or other parts of the body.* After distribution is essentially complete, these curves should parallel the plasma concentration curve, provided that selective localization is not taking place in the site under observation. Assay over the neck also served to confirm the inhibitory effect of Lugol's solution on thyroid uptake of I^{131}.

Renal excretion of radioactivity released from the degraded protein

A consideration which merits attention is the time relationship between the urinary excretion and plasma concentration curves. The extent to which the lag in the urinary excretion of the degraded protein radioactivity affects the curves was evaluated. It can be shown that the rate of appearance of radioactive iodine in the urine rapidly approaches its rate of release from the degraded labeled albumin (Appendix A). Thus, urinary excretion may be accepted as simultaneous with iodo-albumin degradation for the purpose of analysis.

Significance of the "distribution curve"

Analysis of the observed plasma concentration curves reveals a progressively decreasing slope for 4 to 7 days or longer depending on the batch of albumin used. Eventually a straight line could reasonably be drawn through the terminal segments of the curve. Inspection of the distribution curve (Figure 1D) reveals that an horizontal asymptote is reached at the 5th or 6th day. Thus, the decline in the plasma concentration curve after this time is quantitatively accounted for by urinary excretion, indicating that the volume of distribution is no longer increasing. The significance of the horizontal asymptote in the distribution curve is twofold:

1. It offers evidence that during the entire period of study, radioiodine released from degraded protein is almost completely recovered in the urine. If any radioactivity were not recovered, the calculated value for the per cent retained would be higher than the true one by that amount not recovered. The percentage error would then progressively increase during the course of the study producing a negative slope in the curve.

2. It further indicates that any change in the slope of the plasma concentration curve after the distribution curve has become horizontal must be due to a change in the apparent rate of degrada-

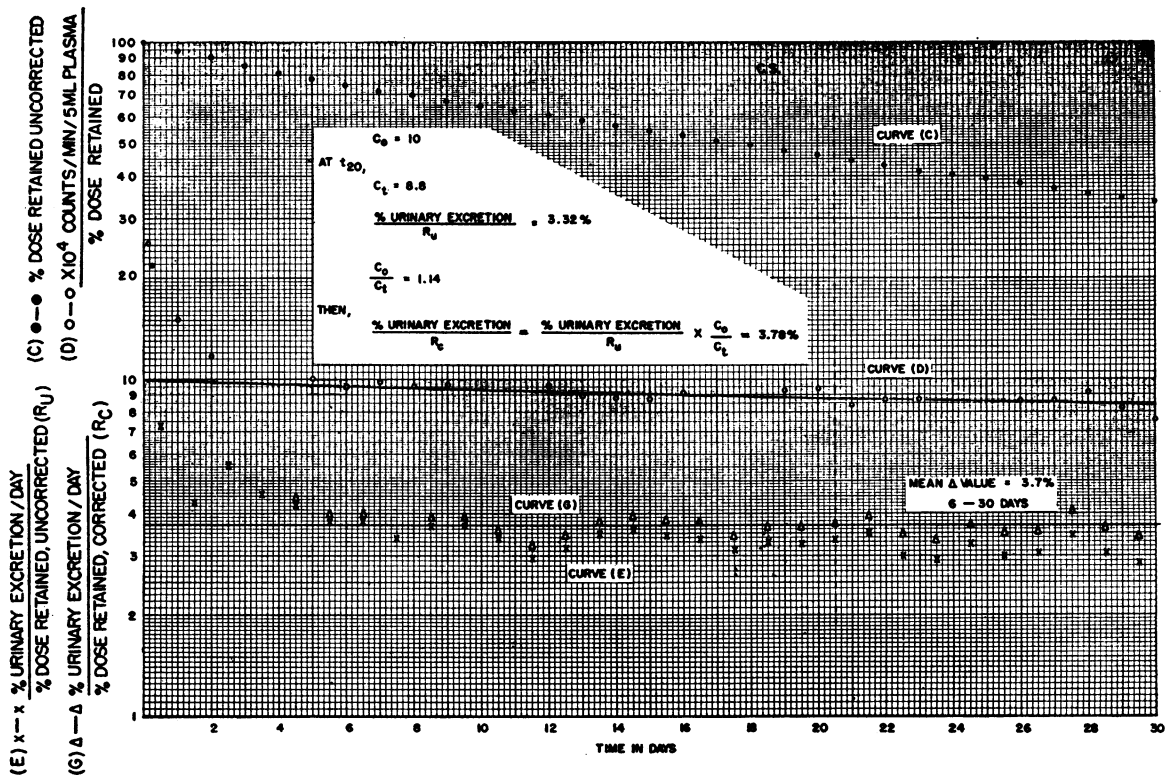

FIG. 2. METHOD OF CORRECTION FOR LOSSES NOT ACCOUNTED FOR IN URINE COLLECTIONS IN SUBJECT C. S. (No. 7)

The calculation for the correction of $\frac{\text{Renal Excretion}}{\text{Per cent Retained}}$ for the 21st day is shown. The mean for the corrected values = 3.7 per cent.

tion and is not to be explained by distribution or storage phenomena.

The observation that urinary excretion accounts for all the degraded protein radioactivity allows for the calculation of the rate of degradation from the urine assays alone. The expression

$$\frac{\text{renal excretion per unit time}}{\text{per cent retained}}$$

defines this rate at any time. Degradation rates determined from two independent sets of data, plasma concentrations and urinary excretions, may thus be compared.

In many cases the distribution curve had a slight negative slope even after apparent distribution equilibrium (Figure 2). Theoretically, this could be explained by continued distribution into less easily penetrated compartments of an albumin pool, or by failure of recovery of all degraded protein radioactivity in the urine, owing either to incomplete urine collection or to extrarenal losses. Continued distribution over the entire period is almost certainly not the explanation, since in that event an approach to a horizontal asymptote rather than a progressive decline would have been observed. In several cases the observations were continued for a period long enough to recover over 85 per cent of the administered doses in the urine when the plasma still contained an additional 5 to 7 per cent. Furthermore, since fecal excretion has already been noted to be insignificant and, in many cases, urinary recovery did account for all losses from the body, it is probable that incompleteness of the urine collections is responsible for the slope observed in these curves. Where such slopes were observed, appropriate correction for the amount not recovered was made as follows (see Figure 2):

Let

C_0 = zero time extrapolation of the final slope of distribution curve,

C_t = point on this slope at time t,

R_u = per cent retained, uncorrected, time t = 100% − (cumulative per cent urinary excretion + per cent withdrawn by blood sampling),

R_c = per cent retained, corrected, time t.

Then

$$R_c = \frac{C_t}{C_0} \times R_u \qquad (1)$$

The renal excretion values may be corrected as follows:

$$\frac{\%\text{ renal excretion}}{R_c} = \frac{\%\text{ renal excretion}}{R_u \times \frac{R_c}{R_u}} \qquad (2)$$

From (1) and (2)

$$\frac{\%\text{ renal excretion}}{R_c} = \frac{\%\text{ renal excretion}}{R_u} \times \frac{C_0}{C_t}$$

II. *Kinetics of distribution into albumin pool*

The distribution curve, rather than the observed plasma concentration curve is used for analysis of the kinetics of distribution in order to exclude the effects of variation in the rates of iodo-albumin degradation during this period. Even here this analysis remains in error to the extent that urinary excretion lags somewhat behind protein degradation (Appendix A). While this could have been more fully accounted for by a separate study of the renal iodide clearance rate, the error involved was not great enough to justify the complicated mathematical treatment required of this refined analysis.

For the purpose of determining more precisely the rates of distribution, 10 to 16 blood samples were drawn during the first 3 to 4 days in about one-fourth of the cases (Figure 3). However, these rates could be grossly evaluated in the remaining cases as well.

Although there may be an unlimited number of exponential components, the distribution curves, in most instances, could be resolved into three major exponential regression rates by conventional analysis (Figure 3). If mechanical mixing in the plasma is considered to be complete at t_0, the form of these curves is then expressed by the gen-

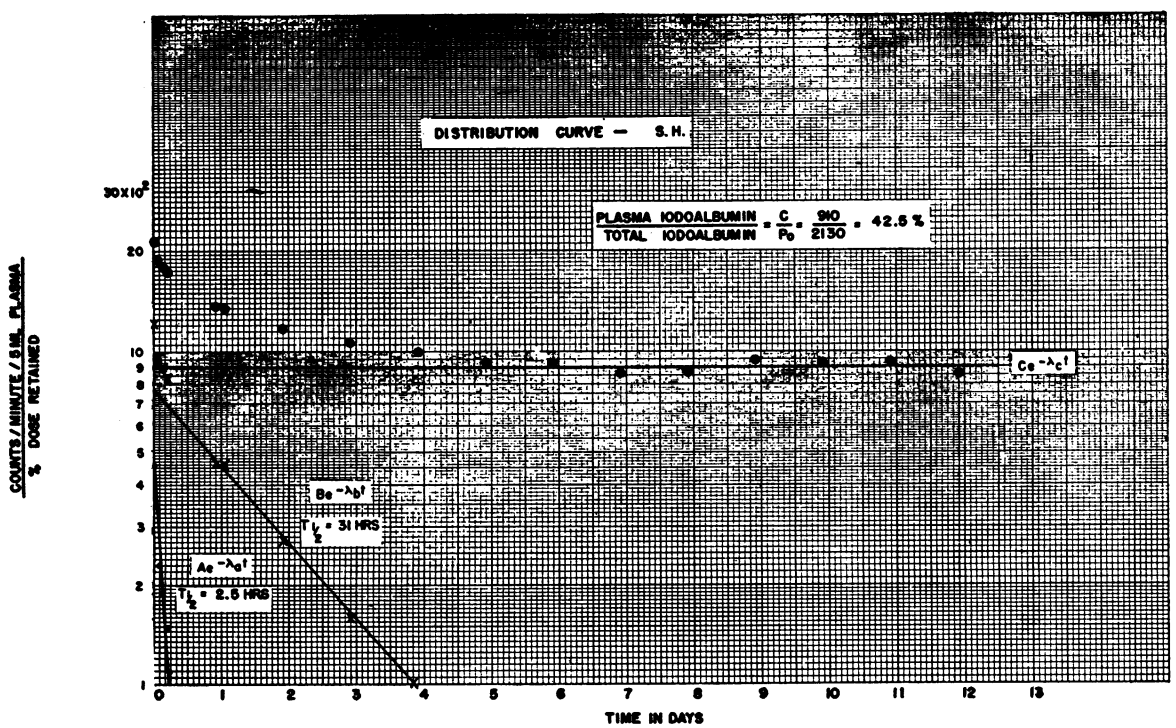

FIG. 3. DISTRIBUTION CURVE IN SUBJECT S. H. (No. 43)

This figure demonstrates the method of analysis for the rates of equilibration between plasma and the "fast" and "slow" extravascular compartments and for the fraction of total iodo-albumin in circulating plasma at equilibrium.

TABLE I

DATA PERTAINING TO DISTRIBUTION AND DEGRADATION OF I^{131} LABELED ALBUMIN

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S			T
											DATA FROM PLASMA CONCENTRATION CURVE										
Method of preparation and lot number	Exp. No.	Subject	Clinical Diagnosis	Age	Weight kg	Mean serum albumin conc. gms/100 ml	Plasma volume ml	% Iodoalbumin extravascular	TEA	TEA / kg	Days to apparent constant slope	Period of observation	$T_{1/2}$	%/day	Albumin degradation gm/day	gm/day/kg	Degradation rate from renal excretion %/day	% / Day Urine excretions— first	three	days	Fraction faster components
																		1	2	3	
A																					
LOTS OF ALBUMIN FREE FROM EXCESSIVE AMOUNTS OF RAPIDLY DEGRADED COMPONENTS																					
B 1-26-3	III A/M	1	J. D.	Parox. Aur. Fib.	53	94	3.84	3280	63	350	3.7	9	32	11	6.3	22.1	.234	6.3	7.3	6.9	6.9	$\frac{1}{5}$
	I.6I A/M	2	M.F.	Diverticulosis	57	52	3.74	2100	60	197	3.8	4	32	16	4.3	8.5	.164	4.0	3.3	2.8	3.4	$< \frac{1}{20}$
		3†	F. A.	C.D.A.	44	64	2.39	3800	61	233	3.6	5	16	>24	<2.9	<6.7	<.105	<0.5	1.0	1.7	1.8	
		4†	Gl.H.	C.D.A.	54	84	2.53	4560	70	385	4.6	9	42	>24	<2.9	<11.1	<.133	1.8	4.1	3.1	2.8	
B 1-7-3		5	J. W.	Rheumatoid arthritis	31	74	3.86	3730	59	351	4.8	6	42	18	3.9	13.7	.185	3.7	5.2	4.9	4.4	0
		6	H.P.	C	64	89	3.38	4100	58	330	3.7	5	42	18	3.9	12.9	.145	3.7	3.8	1.9	3.2	
		7	C.S.	Rheumatoid spondylitis	35	61	4.12	3030	60	312	5.1	5	30	18	3.9	12.2	.198	3.7	6.0	4.0	5.0	
		8	M.B.	Conv. duodenal ulcer	50	57	3.63	2985	59	264	4.6	5	29	17.5	3.96	10.4	.171	4.0	6.0	5.4	4.7	
		9	S.M.	C.D	48	70	2.96	4740	48	270	3.9	16	35	19	3.6	9.7	.139	3.2	3.5	4.4	3.6	
		10	J.B.	Diabetes M.	64	70	3.75 (3.31–4.33)	2850	74	411	5.9	6	35	25	2.8	11.5	.165	2.7	3.5	3.9	4.2	
		11	P. McG	Senile emphysema	67	46	3.97	2880	67	347	7.5	14	34	19	3.6	12.6	.275	3.0	1.9	2.9	4.6	
B 12-2-2		12	J. G.	Buerger's disease	34	66	4.45	2530	55	250	3.8	7	42	15	4.6	11.5	.175	4.6	4.9	6.3	5.3	0–$\frac{1}{5}$
		13†	O.C.	C.D.A.	58	71	2.55	4210	62	283	4.0	3	17	>21	<3.3	<9.4	<.132	<1	1.2	1.7	1.8	
		14†	H.D.	C.D.A.	40	80	2.76	3200	73	327	4.1	14	35	36	1.9	6.3	.079	1.9	2.3	3.4	3.4	
		15	R.T.	C.D.	42	66	2.32	3550	55	183	2.8	12	35	23.6	2.94	5.4	.082	2.85	5.8	4.7	4.3	
A 5-16-2		16	E.C.	Normal	34	74	4.80	2825	65	388	5.3	5	23	18	3.9	15.1	.204	D.I.	4.3	3.5	2.3	$< \frac{1}{10}$
		17	H. W(d)	C.	40	96	4.20	4240	54	386	4.0	10	28	14.5	4.8	18.5	.192	5.1	6.4	5.4	4.6	
A 5-1-2		18	G. S.	Cardiospasm	39	64	4.44	3170	58	335	5.2	4	23	18	3.9	13.1	.204	2.5	4.8	4.4	4.0	$< \frac{1}{10}$
		19	J. O.	Conv. duodenal ulcer	22	68	5.07	3600	54	397	5.8	4	17	13	5.3	21.1	.310	3.5	4.8	5.6	3.5	

B

LOTS OF ALBUMIN CONTAINING APPRECIABLE AMOUNTS OF RAPIDLY DEGRADED COMPONENTS

	20	E.Y.	Senile osteoporosis	69	66	3.89	3050	67	360	3.5	10	30	15	4.6	16.6	.252	D.I.	11.0	7.3	7.6	
	21	S.C.	Conv. duodenal ulcer	38	75	4.44	3600	66	470	6.3	14	25	18	3.9	18.1	.260	3.5	7.7	7.6	5.0	
A 8-28-2	22	M.A.	Post phlebitic syndrome	42	97	4.07	3220	65	375	3.9	8	26	15	4.6	17.2	.178	5.0	9.0	9.5	5.5	1/3
	23	R.G.	Psychoneurosis	32	93	4.50	3710	53	355	3.8	5	14	10	6.9	24.6	.264	5.0	12.3	9.3	6.5	
	24	Ge.H	C. D.	35	89	3.75	4850	54	395	4.4	13	43	18	3.9	15.2	.171	4.7	13.3	9.6	6.0	
	25	F. B.	Rheum. heart dis. compensated	35	77	4.61	3040	63	379	4.9	11	22	12	5.8	21.8	.284	5.0	13.4	8.9	4.9	
ABBOTT A254-13	26	A.J.	Rheumatoid arthritis	58	58	3.70	3700	69	440	7.6	6	22	9.0	7.7	34.8	.583	5.9	12.1	10.3	6.5	1/3
	27	E.M.	C.	45	64	3.55	3410	59	295	4.6	11	22	11	6.3	18.6	.290	5.1	9.4	8.8	6.9	
	28	E.B.	Conv. Scurvy	39	61	4.17	3400	60	354	5.8	15	23	19	3.6	12.9	.212	4.0	9.5	8.5	3.9	
A 5-22-2	29	F.K.	C.*	38	87	5.07	3800	65	550	6.3	9	16	10	6.9	38.0	.440	4.5	6.8	5.5	6.8	1/3
	30	C.R.	Thrombophlebitis	29	74	4.53	3000	59	331	4.5	7	23	11	6.3	20.7	.282	4.5	10.8	9.4	5.4	
ABBOTT A223-2	31	G.A.	P. vera	40	68	4.57	3820	68	546	8.0	5	15	13	5.3	29.2	.430	D.I.	22.4	D.I.	D.I.	1/5
	32	C.D.	P. vera	31	80	3.85	3220	62	326	4.1	14	21	13	5.3	17.8	.222	5.1	15.4	11.2	8.7	
	33	J. McD.	Diabetes M.	34	82	4.69	2850	71	460	5.6	14	21	15	4.6	21.2	.258	3.3	15.6	8.2	3.0	
ABBOTT A216-2	34	F.M.	Conv. duod. ulcer	40	82	4.62	3470	60	400	4.9	17	16	12	5.8	23.1	.282	3.8	21	9.8	5.6	1/3 — 1/2
	35	O.B.	Diabetes M.	42	75	4.38	3940	60	430	5.7	7	21	14	4.9	21.2	.283	4.2	17.7	11.7	5.2	
	36	G.P.	C.	38	85	4.41	3740	58	393	4.6	10	21	13	5.3	20.8	.245	3.6	21	11.3	3.8	
	37	G.O.	C.D.	52	75	3.32	5800	—	—		14	21	24	2.9	—	—	D.I.	12.6	D.I.	D.I.	
ABBOTT A150-4	38	A.S.	Conv. duod. ulcer	42	90	4.50	3670	53	351	3.9	7	14	14	4.9	17.3	.193	5.7	18	11.2	8.4	1/2
ABBOTT A143-2	39	W.S.	Pernicious Anemia	52	68	4.37	2350	70	342	5.0	16	24	14	4.9	16.7	.246	4.8	11	11	6.8	1/4
ABBOTT A137-5	40	J.H.	A.S. Heart Dis. no edema	62	75	4.06	3130	70	424	5.7	9	23	13.5	5.1	21.6	.288	3.6	9.3	7.3	5.0	1/3
	41	A.B.	C.D.	37	70	3.65	4750	52	362	5.2	13	22	16	4.3	15.6	.222	3.3	10.5	8.5	4.9	
ABBOTT A131-3	42	H.H.	C.	34	68	3.51	3660	60	326	4.8	7	21	14	4.9	16.1	.237	4.0	9.2	7.5	6.0	1/5
ABBOTT A111-2	43	S.H.	Ch. glom. nephr.	31	60	4.18	2700	58	268	4.5	9	25	14	3.7Δ	9.9Δ	.165Δ	4.1Δ	8.9	9.0	8.6	1/3
ABBOTT A445-196	44	R.J. (a)	Rheumatoid Arth.	43	85	4.00	3900	61	400	4.7	8	18	13.5	5.1	20.4	.240	4.3	5.8	6.5	4.5	1/5
ABBOTT A065-11	45◆	(b)	" " "	43	85	4.10	3140	62	338	4.0	3	18	9	7.7	26.0	.306	6.1	11.2	9.8	7.6	1/5
ABBOTT A117-2	46◆	(c)	" " "	43	78	3.76	3400	61	327	4.2	5	17	9.5	7.3	23.9	.306	7.3	8.2	11.6	7.3	1/5
ABBOTT A445-142	47	O.S. (a)	" " "	36	70	4.27	3230	56	314	4.5	7	13	17	4.1	12.8	.183	5.0	11	7.1	5.5	1/5
	48	F.Z. (a)	" " "	52	62	3.62	3620	54	285	4.8	5	13	15.5	4.5	12.7	.205	3.8	7.3	9.6	6.0	
ABBOTT A446-178	49◆	O.S. (b)	" " "	36	70	4.26	3130	55	295	4.2	4	16	13	5.3	15.7	.224	5.5	8.6	7.3	6.5	1/10
	50◆	F.Z. (b)	" " "	52	62	3.62	3690	51	272	4.4	5	10	11	6.3	17.1	.276	5.6	8.6	7.4	6.1	
ABBOTT A446-128	51	H.W. (a)	C.	40	98	3.50	4360	50	305	3.1	6	15	13.5	5.1	15.6	.160	7.8	12	6.7	11	1/5
ABBOTT A445-147	52	(b)	C.	40	96	3.50	4580	46	298	3.1	10	19	14	4.9	14.7	.153	4.0	6.3	6.2	6.0	1/5
ABBOTT A014-2	53	(c)	C.	40	96	3.70	4450	53	351	3.7	6	30	11	6.3	22.1	.230	5.5	4.8	9.4	5.5	1/10

C—Hepatic cirrhosis, compensated.
CD—Hepatic cirrhosis, decompensated without detectable ascites.
CDA—Hepatic cirrhosis, decompensated with ascites.
conv—Convalescing.
D.I.—Data insufficient.
†—Ascites increasing during observation period.
‡—Ascites not increasing during observation period.
*—In spite of repeated high serum albumin concentration, diagnosis proven by biopsy.
Δ—25 per cent of urine radioactivity consistently in protein precipitable fraction—degradation rates calculated from non-protein bound radioactivity excretion.
⊖—Under Cortisone therapy during these studies. Because of uncertain reliability of these lots, the apparent increase in rate of degradation, as compared with control studies in the same subjects, while suggestive, is not considered established.

eral formula:

$$P = Ae^{-\lambda_a t} + Be^{-\lambda_b t} + Ce^{-\lambda_c t}$$

where

$P = \frac{\text{Plasma concentration}}{\text{Per cent retained}}$

λ_c[5] = Rate constant due to incomplete urinary recovery of degraded protein radioactivity. In many cases this equalled zero, as noted above.

λ_a and λ_b = Rate constants determined by transfer of tagged albumin between plasma and two apparent extravascular compartments. λ_a is calculated from the slope of the terminal straight line phase of the curve. Subtraction of the values of this extrapolated line from the corresponding values of the curve produces a new curve. λ_b and λ_a are then determined in a similar manner from successive resolution of the curves so produced.

A, B and C = Coefficients obtained from the vertical axis intercepts of the regression lines, $Ae^{-\lambda_a t}$, $Be^{-\lambda_b t}$, $Ce^{-\lambda_c t}$, respectively.

At $t = t_0$, $P = P_0$. Then,

$P_0 = A + B + C$,

$\frac{C}{P_0}$ = fraction of total iodo-albumin in plasma at distribution equilibrium,

$\frac{A + B}{P_0}$ = fraction of total iodo-albumin in spaces other than plasma at distribution equilibrium.

Total plasma albumin = albumin concentration × plasma volume.

If iodo-albumin is assumed to exchange with endogenous albumin, then,

Total exchangeable albumin (TEA) = total plasma albumin $\times \frac{P_0}{C}$.

Values for $\frac{A + B}{P_0}$ are given in column I of Table I. The mean value for compensated cirrhotic and non-cirrhotic subjects was 59.5 per cent. The somewhat higher percentage (mean 66.5 per cent) in the presence of ascites was to be expected. In case H. D. (No. 14), paracentesis on the last day of observations revealed that approximately 60 per cent of the total iodo-albumin was present in the 19 liters of ascitic fluid removed, and in case Gl. H. (No. 4) 42 per cent was present in 15.5 liters. In case F. A., (No. 3) 6.5 liters of ascitic fluid contained 16 per cent of the iodo-albumin in the body.

Values for TEA are given in column J of Table I. In many of the cirrhotic subjects TEA was not significantly lower than in non-cirrhotics. In these cases an increase in plasma volume or the presence of fluid accumulations compensated for the low serum albumin concentrations.

Extravascular compartments

In all but a few of the studies, two greatly different rates of distribution were observed with mean half times of approximately 3 and 24 hours. For lack of more precise information as to their localization, these two extravascular compartments are merely termed "fast" and "slow." In the possibility that these compartments were not diffusely distributed throughout the body, *in vivo* assays were performed in an attempt to localize sites of build-up of radio-activity.[6] However, no such sites were discovered by assays over the heart, lungs, liver, spleen, neck or thigh taken 15 minutes after injection and at frequent intervals thereafter. Since in the liver and spleen areas, the counter "saw" a relatively large amount of blood compared to extravascular tissues, these curves approximately paralleled the heart and plasma curves during the distribution phase (Figures 4, 7). On the other hand, the neck and thigh curves followed more closely the curve of per cent dose retained (Figures 4, 1). Only over the mid-abdomen in some patients with ascites was there any suggestion of a "build-up" curve indicative

[5] Theoretically, λ_c is not a constant whether the loss is a single loss or distributed throughout the course of the experiment. However, in practice, the losses were of such slight degree that λ_c could be treated as a constant without significant error.

[6] It is postulated that these two compartments communicate directly with the plasma rather than end-wise with each other although it is to be appreciated that distinction between these two arrangements cannot be made from the plasma observations alone.

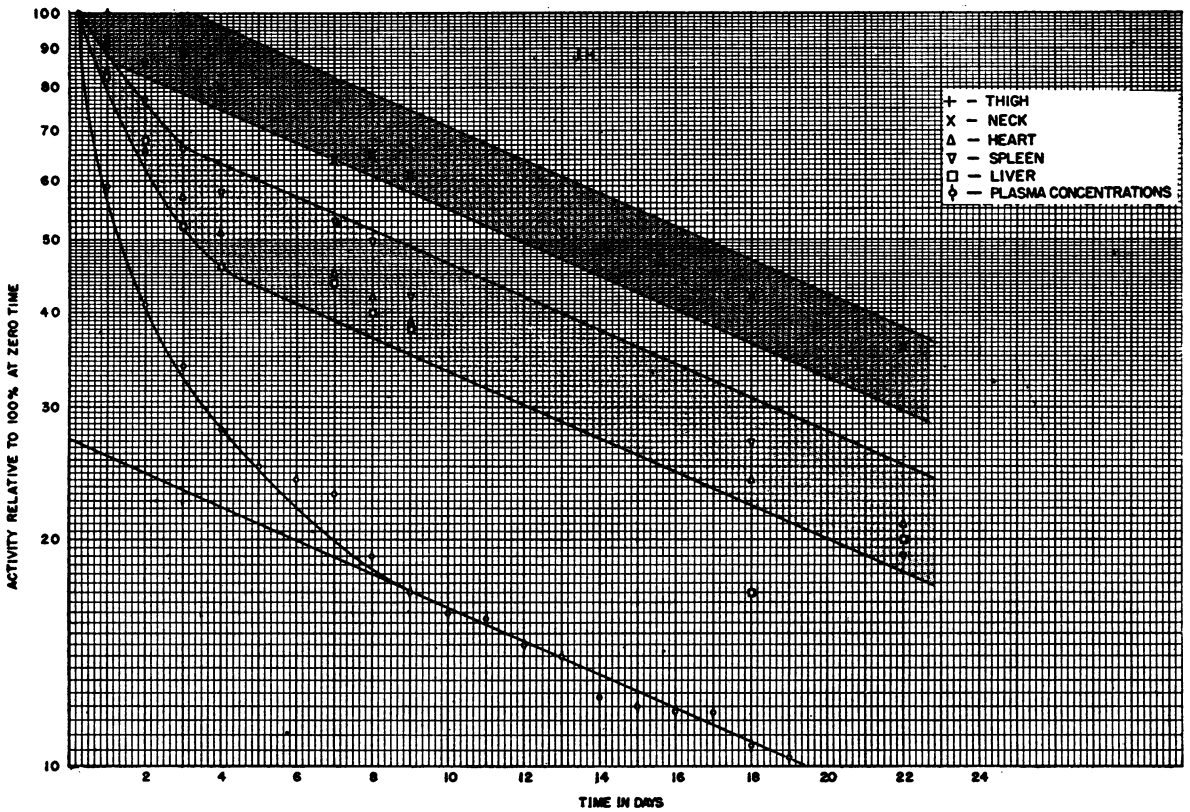

FIG. 4. SUBJECT J. H. (No. 40). TIME COURSE OF RADIOACTIVITY IN PLASMA AND IN VARIOUS BODY SITES

The stippled envelopes emphasize differences between areas which are exceptionally vascular and those which are not.

of localization. After distribution equilibrium was reached, the slopes of all these curves were approximately the same as that of the plasma concentration curves.

Removal of iodo-albumin from tissue sites

It is relevant to recall the studies of Cope and Moore (11) in dogs. By direct lymph analysis they demonstrated a much more rapid equilibrium between plasma and thoracic duct lymph than between plasma and lymph from the cervical and leg chains. Some evidence in human subjects is obtained from the rates of disappearance of tagged albumin from sites of injection into muscle, skin and subcutaneous tissue of the lower extremity. The half times for removal from these sites in 16 experiments ranged from 13 hours (Figure 5) to 72 hours (previously published figure [9]) which are consistent with the "slow" rate of exchange determined by the plasma studies.

From the observations presented here, no conclusions can be derived as to the presence of an intracellular phase except to note that *in vivo* assays failed to give evidence for localization in the liver as had previously been demonstrated for tagged modified human globin (9).

Iodo-albumin transfer between plasma and ascitic fluid

In one subject (Gl. H.), repeated sampling (20 to 30 ml.) of ascitic fluid was performed. During the period of this study, the ascitic fluid volume was increasing only slightly as judged by observation and daily weighings. Plasma and ascitic fluid distribution curves are shown in Figure 6. The ascitic fluid concentrations appeared to approximate a single exponential growth curve with a rate constant of 0.0150 per hour ($T_{1/2}$ = 46 hours). Theoretically, however, exchange between plasma and extravascular pools other than

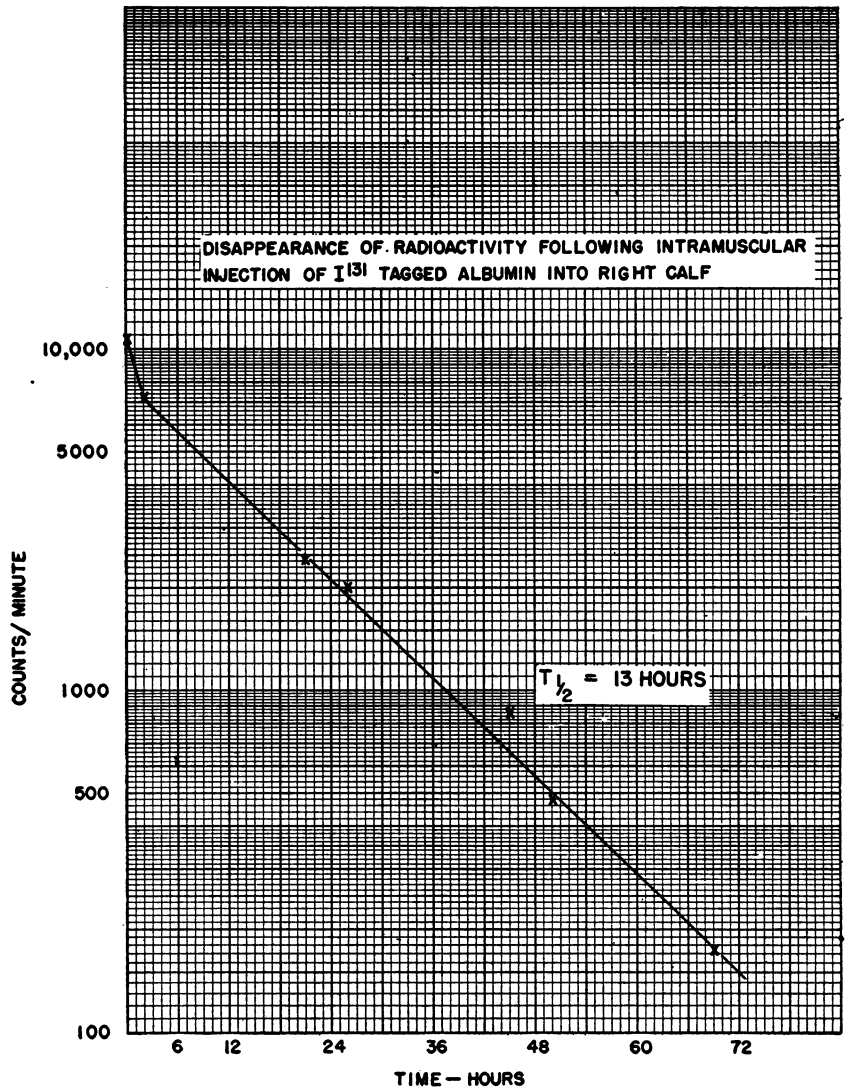

FIG. 5. DISAPPEARANCE OF IODO-ALBUMIN FROM MUSCLE

This was the shortest half-time observed. In most experiments the half-times for removal from skin, muscle or subcutaneous tissue of the lower extremity ranged from 18 to 36 hours.

the peritoneal cavity makes the situation much more complex. Because of this and the lack of appropriate observations of exchange rates and volumes of these compartments, the analysis of the kinetics of mixing was simplified by assuming a single rate of transfer into ascitic fluid. The dynamics of albumin exchange across the peritoneal membrane were evaluated in this manner under each of two extreme conditions (Appendix C). The calculations reveal that approximately 0.8 gram albumin passed the peritoneal membrane in each direction per hour.

III. *Degradation of tagged albumin*

As previously indicated, the slope of the plasma concentration curve following the distribution phase is a function of the rate of degradation of iodo-albumin. However, this portion of the curve was a straight line in only a small number of cases. These cases were limited almost exclusively

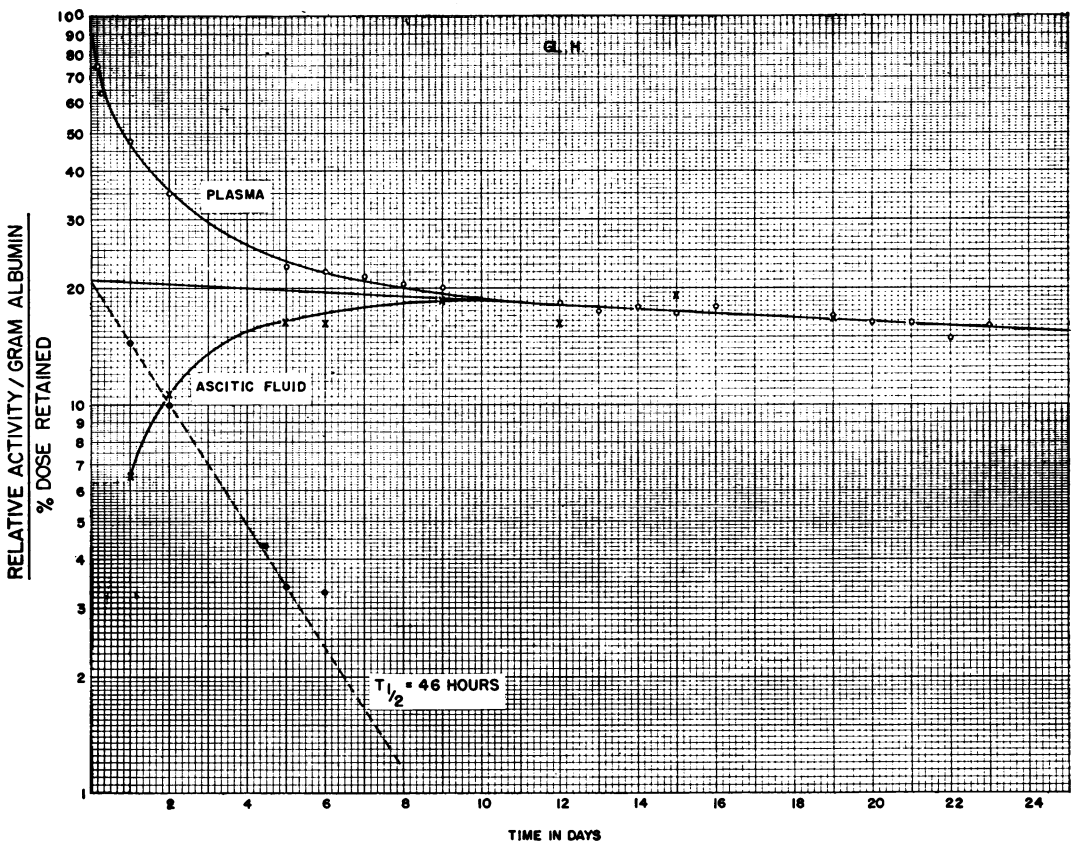

FIG. 6. CURVE OF EQUILIBRATION BETWEEN PLASMA AND ASCITIC FLUID IN SUBJECT GL. H. (No. 4)

Equal specific activity in plasma and ascitic fluid was apparently reached in 10 days. The dotted line is a plot of the differences between the extrapolated equilibrium value and the observed ascitic fluid concentrations.

to lots prepared by method B. In these, the renal excretion curve, after the fifth or sixth day, was also a straight line and had a slope similar to that of the plasma concentration curve (Figure 7). The curve of $\frac{\text{renal excretion}}{\text{per cent retained}}$ remained at a horizontal level after distribution equilibrium was reached. The rates of degradation of the iodoalbumin as determined from the slopes of the plasma concentration curves were in close agreement with the values obtained from the horizontal portions of the $\frac{\text{renal excretion}}{\text{per cent retained}}$ curves (Table IA). In these cases, therefore, the observations are consistent with the interpretation that nearly all of the labeled albumin was being degraded at a single constant rate, which could be independently derived from plasma or urine analyses. During the phase of distribution, $\frac{\text{renal excretion}}{\text{per cent retained}}$ was slightly higher than during the subsequent flat portion of the curve.

Unfortunately, however, these conditions did not obtain in the majority of cases. The following characteristics of the post distribution segments of the curve were frequently observed (Figure 1). The slope of the plasma concentration curve revealed a gradual decrease. The slope of the renal excretion curve was greater initially but tended to approach that of the plasma concentration curve terminally. The curve of $\frac{\text{renal excretion}}{\text{per cent retained}}$ did not approach a horizontal level for some time after distribution was complete but rather showed higher levels in the initial portions of the post distribution segments. In these cases, therefore,

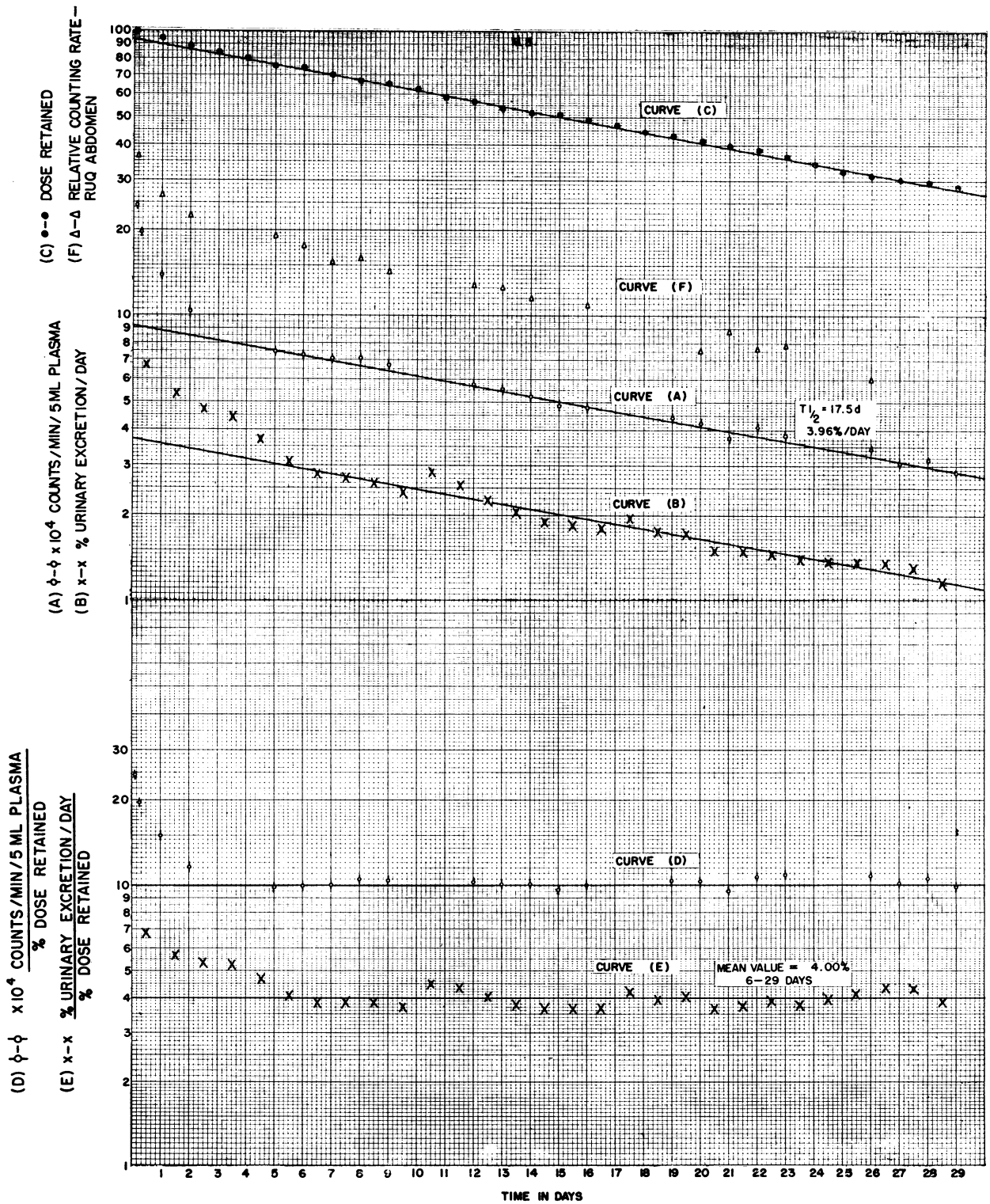

FIG. 7. SET OF CURVES DERIVED FROM DATA IN SUBJECT M. B. (No. 8)

In all cases in which this lot (B 1–7–3) of albumin was used, a single rate of degradation was observed.

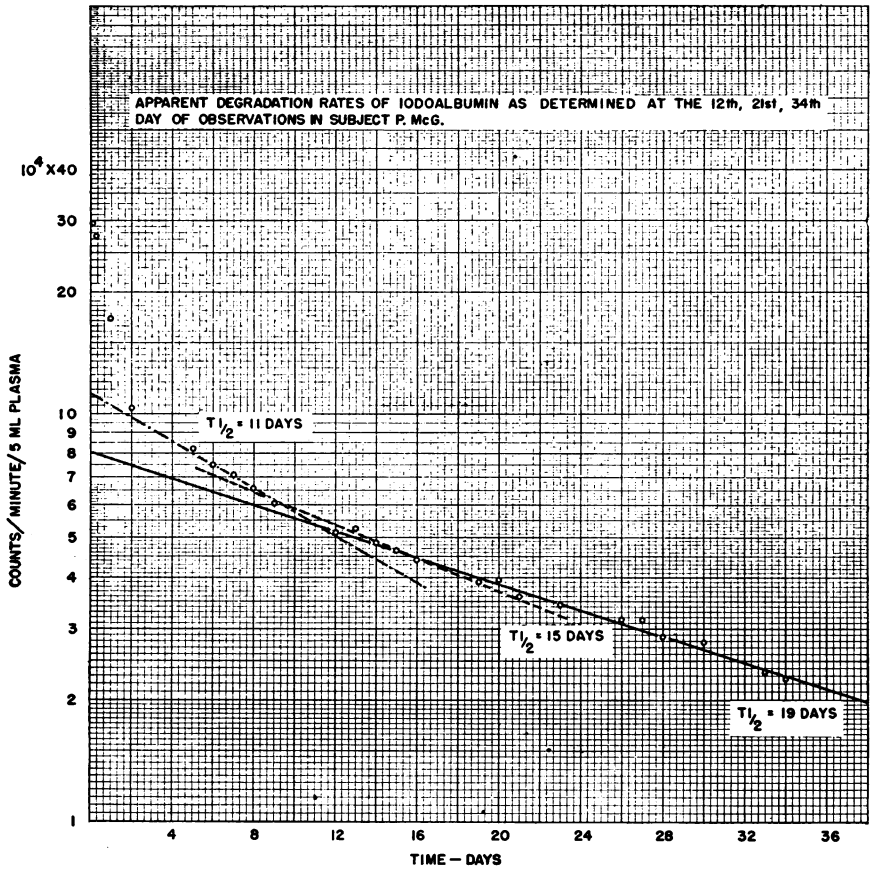

FIG. 8. PLASMA CONCENTRATION CURVE IN SUBJECT P. McG. (No. 11)
Had this experiment lasted only three weeks or less, a spuriously rapid degradation rate would have been obtained.

it is evident that degradation of the iodo-albumin was not proceeding at a single constant rate, but that there were several components being degraded at different rates. Theoretical curves for plasma concentration and renal excretion, when constructed for a two-component system, exhibit just such characteristics as have been observed in these studies (Appendix B).

As an example of the difficulties encountered in attempting to evaluate the rate of tagged protein degradation from most plasma concentration curves, the best straight line was drawn through the observed points in various phases of the study in subject P. McG. No. 11 (Figure 8). As the experiment proceeded the rate appeared progressively slower until the last half time of 19 days (3.6 per cent per day) was obtained for the 14th to 34th days after administration of I^{131} tagged albumin. In many cases it was two weeks or longer (Table I, column L) before the degradation rate became constant. Thus a minimum of three to four weeks was often required for evaluation of this final rate.

The fractional quantity of the last (slowest) component was estimated by dividing the zero time extrapolation of the final component of the plasma concentration curve by the zero time extrapolation of the terminal segment of the distribution curve (Figure 1). The constancy of the rate of degradation of the slower component during distribution may be seriously questioned. However, since this fraction of the iodo-albumin was degraded at a relatively slow rate, the error was small and the values obtained were useful as rough approximations. The observations did not permit the more refined approach utilized in the analysis of the distribution curves. It was found that the fraction of iodo-albumin which was

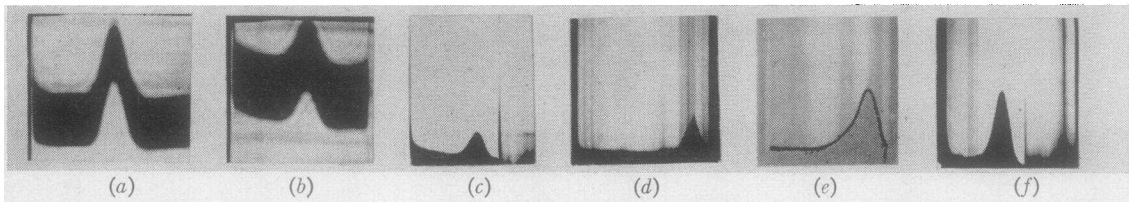

FIG. 9.

Ultracentrifugal patterns of some lots used in this study: (*a*) and (*b*)*. Uniodinated albumin (*a*) compared with iodinated albumin (*b*) from lot Abbott A-143-2 used in case W. S. (No. 39). Although the latter material showed 50 per cent rapidly degraded components, there are no significant differences between the two patterns and the sedimentation constants are identical ($S_{20} = 4.3$ Svedberg units); (*c*) Albumin prepared by Method A (lot 5-22-2) used in cases (Nos. 28, 29, 30) in which about one-third of the material was rapidly degraded. $S_{20} = 4.6$ S.U. units. There are no abnormalities in the sedimentation pattern; (*d*) Abbott albumin B-192-37. Although there is only one sedimenting peak recorded, the sedimentation constant ($S_{20} = 5.7$ S.U.) is higher than that of "normal" albumin (ca. 4.7). This material gave high urine excretions over the first 24 hours (Table II); (*e*) Lot B 1-26-3 prepared with 11.1 I atoms per molecule protein and used in subject J. D. (No. 1). The "shoulder" on the "heavy" side indicates the presence of heavier polydisperse material in the preparation. In addition the sedimentation rate of the major component ($S_{20} = 6.2$ S.U.) is higher than normal, indicating appreciable alteration; (*f*) Lot B 1-26-53 prepared with 1.6 I atoms per molecule protein. This material which was used in subject M. F. (No. 2) was prepared identically with that in (*e*) except for the lower iodine: protein ratio. The pattern and sedimentation constant ($S_{20} = 5.0$ S.U.) are normal; used in subject M. F. (No. 2). Direction of sedimentation to the left in all frames.

* We are indebted to Dr. Sam Sorof, Vet. Adm. Hosp., Bklyn., N. Y., for performing these two analyses.

rapidly degraded was characteristic of the lot of iodo-albumin used (Table I, column T). In general, the lots prepared in the laboratory by Method B were the most satisfactory and those obtained from Abbott Laboratories the least so with respect to minimizing the portion of these rapidly degraded components. The presence of large amounts of these rapid components was evident in the high urine excretions of radioactivity during the first few days (Table IB, column S). From experience gained with the different lots it became clear that when more than 6 to 7 per cent had been excreted during the first 24 hours, it was likely that more than one component would subsequently be observed. However, even a lower excretion during the first day did not always exclude the presence of more than one component. It may again be emphasized that ultracentrifugal analysis often failed to detect such abnormalities in the various lots (Figure 9 b and c). However, a more recent lot of Abbott I^{131} tagged albumin has shown slight abnormalities in the ultracentrifuge (Figure 9 d). This and other recent lots have also shown extremely high renal excretions for the first 24 hours (Table II). These cases have not been included in the present study.

TABLE II

Renal excretions after administration of some recent lots of iodo-albumin supplied by Abbott Laboratories

Lot No.	Date shipped	Subject	Renal excretion % of dose administered	
			1st 24 hours	2nd 24 hours
B-42-6	7-24-52	J. S.	9.4—not dialyzed before administration	
B-71-25	8-21-52	F. M.	13.1	
B-156-2	11-26-52	S. F.	18.8	
B-193-37*	1-8-53	C. C.	13.3	
		E. K.	19.2	
B-210-40	1-30-53	L. I.	10.7	
		I. B.	6.2	
B-221-27	2-17-53	F. B.	22.7	
B-233-2†	2-20-53	H. M.	4.1	4.2
B-241-34	3-2-53	E. I.	17.4	

* High sedimentation constant on ultracentrifugal analysis (Figure 9d).

† Special experimental lot.

Degradation rate of final (slowest) component of iodo-albumin

Where observations were continued over a period sufficiently long for the faster components to appear to be almost completely degraded, the rate of degradation of the final component was almost always less than 5.5 per cent per day ($T_{1/2} = 12.5$ days). Subject J. D. (No. 1) who received albumin with an average of 11 Iodine atoms per molecule is an exception. This case is discussed in greater detail below. In the case of those experiments with lots containing a single detectable component or only a small fraction of faster components and where observations over a four week period or longer were available, the rates were even much slower, being of the order of 4 per cent a day or less ($T_{1/2} > 17$ days) (Table IA). In these cases, too, the degradation rates determined independently from the plasma concentration and $\frac{\text{renal excretion}}{\text{per cent retained}}$ curves were in excellent agreement. If the daily degradation of endogenous albumin is accepted as the product of total exchangeable albumin and the degradation rate ($.693/T_{1/2}$ days) of iodo-albumin, then in this group, the albumin "turnover" per day ranged from 5.4 to 21.1 grams (.079 to .310 gram per kilogram per day) (Table IA). The values for cirrhotic patients (range .079 to .192; mean .126 gram per kg. per day) were lower than those for non-cirrhotic subjects (range .164 to .310; mean .205 gram per kg. per day). Even if calculated on the basis of "dry weights," the calculated "turnover" values would reveal low figures for the cirrhotic subjects. In two of the cirrhotic patients who died in cholemia and hepatic decompensation (O. C., No. 13 and F. A., No. 3) the rates of degradation could not be determined precisely from the plasma concentration curves since these patients were continually accumulating ascitic fluid during the study. This led to a decay in the plasma concentration apparently in excess of the actual rate of degradation. However, the renal excretion values indicated extremely low turnover rates.

In one other cirrhotic subject (Gl. H., No. 4) with increasing ascites, the renal excretion also showed lower values than the rate determined from the plasma concentration curve. Although TEA must have been increasing slightly during the period of study, if the net increase in ascitic fluid albumin is calculated as the product of TEA at distribution equilibrium (385 grams) and the difference between the plasma concentration decay rate (2.9 per cent per day) and the degradation rate ($\frac{\text{renal excretion}}{\text{per cent retained}} = 1.8$ per cent per day), a mean value of 4.2 grams per day is obtained. This agrees fairly well with the value (3.6 grams per day) derived by dividing the total albumin (214 grams) removed by paracentesis at the end of the study by the number of days elapsed since the previous tap (59 days). During a period of 80 days prior to the study this patient had been relieved of 585 grams of albumin by repeated paracenteses. The losses (7.3 grams per day) by this route alone were almost as much as the total turnover values in some non-cirrhotic subjects in this group. This indicates the tremendous additional load imposed upon the albumin productive capacities of the liver by repeated withdrawal of ascitic fluid in these subjects. It is conceivable that, in some cases, such practice might demand the synthesis of even greater than normal amounts of albumin.

In most of the studies with the remaining lots, insufficiently long observation periods and the presence of rapidly degraded components combined to produce apparently shorter half time in the plasma curves and poor agreement between rates determined from plasma and urine curves. In most of these cases the turnover rate of the final component as determined from the urine excretions was lower than the rate derived from the plasma concentration curves essentially because the constructed slopes of the terminal portions of the plasma curves were more prejudiced by earlier points which included varying amounts of the faster degraded components. The turnover rates given in Table IB, therefore, are, in general, spuriously high and represent only maximum values.

In some lots the rate of degradation of these faster components was only slightly greater than that of the slowest component (*e.g.*, lot B12–2–2). In others (*e.g.*, lot No. Abbott A 216–2) probably due to a more marked degree of altera-

tion of the protein, extremely rapid breakdown of a fairly large fraction was obvious within the first day or two (see Table IB, column S). Since the slope continued to change for a few weeks, however, it is likely that a more or less continuous spectrum of components degraded at different rates was present. For this reason it is questionable whether a "final component" in these cases was ever really observed. Furthermore, because of inadequate observation periods, the calculated "fraction of faster components" is, in some cases (*e.g.,* Abbott A 014–2), falsely low, owing to a spuriously steep slope in the plasma concentration curve extrapolating back to an apparently high zero time value.

Review of the entire mass of data thus leads to the conclusion that the apparent rates of degradation depended in large part on the particular lot of iodo-albumin used. However, with what were considered the best batches of material, the rates were appreciably less than 5 per cent per day.

Effect of overiodination of albumin on the degradation rate

In order to investigate the possibility that differences in the rates of degradation were related to the degree of iodination of the protein molecules, two portions of iodo-albumin containing an average of 1½ and 11 iodine atoms per molecule of protein, respectively, were prepared identi-

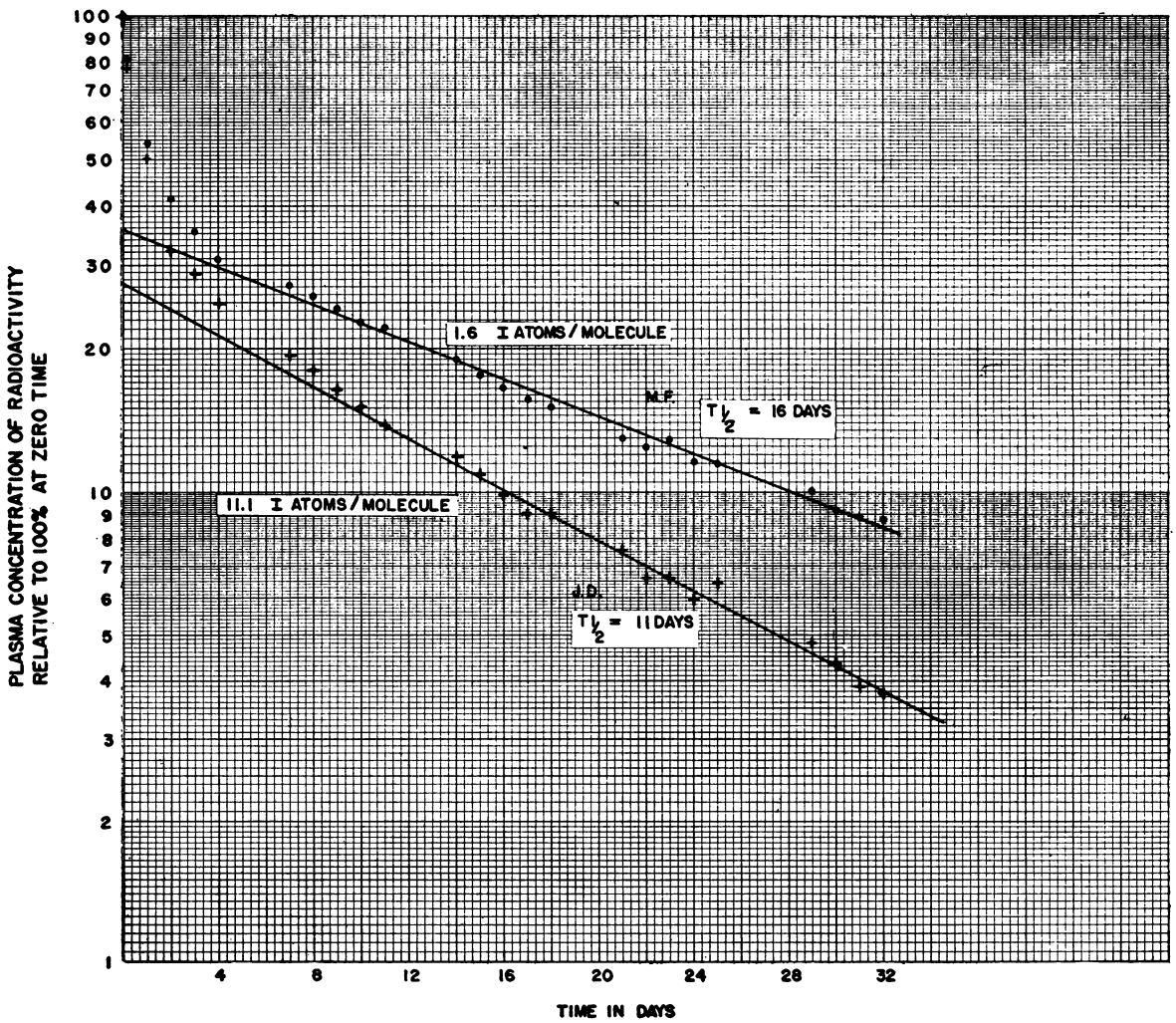

FIG. 10. PLASMA CONCENTRATION CURVES IN SUBJECTS M. F. (No. 2) AND J. D. (No. 1) SHOWING RELATIVELY RAPID DEGENERATION IN THE MORE HEAVILY IODINATED PROTEIN

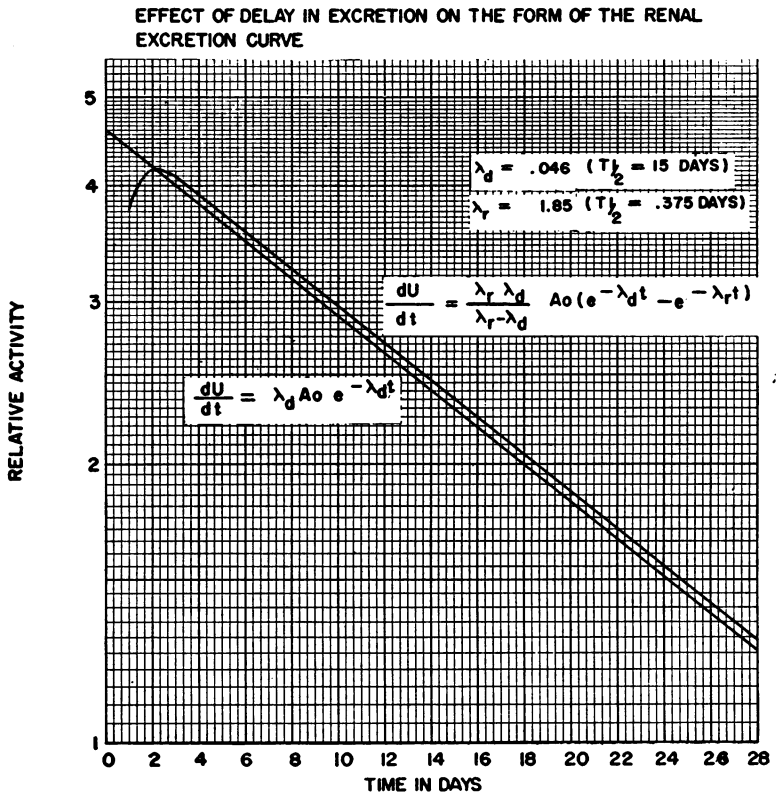

FIG. 11

See Appendix A for interpretation.

cally [6] from the same batch of protein and each was administered to a non-cirrhotic subject (M. F., No. 2 and J. D., No. 1, respectively). The plasma concentration curves (Figure 10) clearly indicated a difference in the rates of degradation, the more highly iodinated material being degraded more rapidly. Ultracentrifugal analysis revealed marked alterations in the sedimentation of the latter material (Figure 9 e). In spite of these obvious changes, however, the rate of degradation was not inordinately fast ($T_{1/2}$ = 11 days) and suspicion of such marked alterations would not have been aroused by the renal excretions (Table IA, column S). Because of the exceptional care taken in the preparation of this lot, fairly uniform iodination of the protein molecules must have been obtained and the material, therefore, behaved biologically in a more or less uniform manner.

In view of the very rapid combination of elemental iodine with protein in the process of iodination, it is quite conceivable that during preparation, some portion of the protein may become very heavily iodinated even when the average iodine: protein ratio is no more than 1 to 2 atoms per molecule. These very heavily iodinated molecules, containing a large portion of the total radioactivity may yet comprise only a very small fraction of the total protein. Thus, alterations in the tagged protein may fail to be detected in the ultracentrifuge. In the case of the albumin labeled with an average of 11 I atoms per molecule, abnormality was readily evident in the ultracentrifugal pattern probably because much of the protein itself must have been altered at such a high average iodination level. Where a low average iodination level was present, as in all the other lots used in this study, it was repeatedly demon-

[6] The iodine solution was slowly added to the albumin solution with continuous mixing. After an amount calculated to produce a ratio of 1.5 I atoms per molecule had been added, one-half of the protein solution was removed and the remainder was then more heavily iodinated in the same fashion. The final ratios as determined from nitrogen analysis were 1.58 and 11.1 I atoms per molecule albumin, respectively.

strated that a normal sedimentation pattern did not insure biological integrity of the tagged moiety.

If uneven iodination were responsible for the variability of degradation rates, extremely high iodine: protein ratios must have been present in some molecules of many of the lots since even the 11 I atoms per molecule iodo-albumin did not lead to such high renal excretions in the first few days as were observed in most of the Abbott preparations. The inference that, in these lots, a large part of the radioactivity was bound to a small moiety of the albumin is consistent with the frequent failure to identify abnormalities on ultracentrifugation. It is possible, therefore, that the apparent multiple iodo-albumin components frequently observed were merely albumins with different degrees of iodination although additional alterations during isolation or iodination cannot be excluded. These considerations do not absolutely negate the possibility that the presence of multiple components in "native" albumin also contributed to these variations, although the absence of more than one component in at least one lot (No. B1-7-3) offers no support for this alternative.

DISCUSSION

The biological behaviour of the isotopically labeled simple ion or chemically defined compound may be accepted, in general, as reflecting that of the native unlabeled species except for consideration of radiation and so called isotope effects. In the case of complex substances such as proteins, however, *in vitro* labeling procedures may induce subtle alteration beyond the scope of detection of available physical and chemical techniques. The validity of the tracer thus remains suspect. Furthermore, its identical behaviour to that of the native substance in certain biological systems (*e.g.*, immunological) does not, in itself, warrant the assumption of such similarities in other systems (*e.g.*, hormonal activity or response to catabolic processes). For example, the precipitation by specific antibody of bacterial toxin and toxoid hardly establishes their identical toxicity. Similarly, the parallel rate of disappearance of I^{131} labeled and native human serum albumin from the blood stream of the rabbit (3), to which they are both foreign proteins, does not necessarily insure similar behaviour in the human subject. Indeed, iodo-albumin is not itself a protein native to man. Conversely, however, it has been repeatedly demonstrated elsewhere (3, 4), as well as in the present study, that obvious alteration does lead to a more rapid rate of disappearance and it probably can be accepted that the slowest rate of disappearance most nearly approaches that of the native albumin.

The difficulty in determining whether a more rapidly degraded component is an altered protein or one of several normal albumin fractions is merely one of a number of problems in the interpretation of such data as are reported here. A principal question concerns the process of degradation. The implication that the rate of disappearance of radioactivity from the body reflects the rate of dissolution of the entire albumin molecule must be avoided. The observations presented here and in available published reports offer no information as to the mechanism of splitting of the I^{131} albumin linkage in the body. Although, more probably, the iodotyrosine fragments are separated by peptide bond hydrolysis, there is, as yet, no evidence against the possibility that simple deiodination alone occurs *in vivo* in this abnormal protein. Even if hydrolysis alone were demonstrated, the rate of this process is not manifestly the same as the turnover of the normal tyrosine fragments. Furthermore, if there is any turnover within the intact protein, then the rates for the various amino acid residues may be quite different. In this event the term "albumin turnover" would be quite meaningless unless qualified by reference to a specific amino acid. Multiple isotope labeling in different amino acid positions may clarify some of these problems.

Where N, C or S isotopes are used it is reasonable to expect an error in the labeled amino acid turnover rates obtained because of reutilization of some fraction of the isotope released by protein degradation. This particular error is probably of no significance in data obtained from I^{131} labeled protein studies, especially in the presence of thyroid blockage. In the absence of thyroid binding, I^{131} administered as iodide (12, 13) or as di-iodotyrosine (14) is almost quantitatively recovered in the urine within 48 to 72 hours. Further evidence is offered by the recovery of all detectable plasma radioactivity in the electrophoretically separated albumin fraction.

The final question concerns possible differences

among the albumins synthesized by the body in health and various disease states, and whether pooled serum albumin is handled in the same fashion as endogenous protein. In cirrhosis and the nephrotic syndrone, Luetscher (15) has demonstrated an alteration in the ratio between the two components of human serum albumin normally demonstrable in the electrophoresis apparatus at pH 4.0. Therefore, isolation and labeling of the subject's own albumin would be a more valid technique, but the accumulation of any considerable mass of data by this method represents a formidable undertaking.

In view of these considerations, it is advisable until further information is available, to interpret the observations presented here, and in similar studies, simply in terms of separation of the radioactive label from the protein, especially when dealing with degradation rates. In the case of distribution phenomena it is more probable that the behaviour of I^{131} labeled albumin closely reflects that of the native protein. The distribution studies have clearly demonstrated that mixing in the entire exchangeable albumin pool is not essentially complete until 4 to 7 days after administration and may be even more prolonged in subjects with massive ascites. Schoenberger, Kroll, Sakamoto, and Kark (16) studied the rate of appearance of I^{131} labeled albumin in the ascitic fluid of a cirrhotic subject in a manner similar to that reported here. They found that equilibrium between plasma and ascitic fluid took 38 and 47 hours in the two studies performed on their patient. However, at the time of the studies, their subject had ascitic fluid volumes of only 890 and 1950 ml., respectively. Their results, therefore, are not inconsistent with our findings of 9 to 10 day equilibration periods in subjects with ascitic fluid compartments of 7 to 19 liters. In fact, the albumin transfer rate across the peritoneal surface was even greater in our subject than in theirs.

Previous studies (3, 16, 17), which have dealt only with observation periods of 11 to 15 days or less, have reported significantly faster degradation rates than in the present study. In general, these rates were calculated from plasma points taken as early as the third day. Inspection of the published curves in these reports indicates that a possible flattening out of the curves has been ignored. Since distribution is usually not complete for 4 to 7 days, inclusion of these early points results in an apparent but erroneous fast rate of degradation. Furthermore, the presence of slower components is likely to escape detection unless observations are carried out for a period of time sufficient to account for the excretion of most of the administered dose.

Although the experiments with heavily iodinated albumin may explain the presence of multiple components with different degradation rates, there is evidence to support the concept of more than one native serum albumin. The presence of two electrophoretically distinct albumin components at pH 4.0 observed by Luetscher (15) has been confirmed by Cohn, Hughes, and Weare (18). According to Hughes (19, 20) more than half of normal serum albumin is mercaptalbumin with a single available sulfhydryl group per molecule, the remaining serum albumins appearing to have no available sulfhydryl groups. The labeling of these various fractions should be of interest.

Whether the multicomponent systems observed in the present study were related entirely to artifacts produced by iodination or in part to different native albumins, the major component in the best preparations was degraded at the slowest rate which approximated a half time of 15 to 23 days. These values are consistent with the results obtained by biosynthetic techniques (1, 2). However, in light of the previous discussion, conclusions based on this similarity in rates must be guarded. The marked variability of degradation rates observed in the different lots of radioiodinated albumin further emphasizes the need for a conservative approach. It is hoped that further studies will be directed along the lines of these inquiries before precarious deductions are formulated on the basis of I^{131} labeled protein "turnover" rates.

SUMMARY AND CONCLUSIONS

1. I^{131} labeled human serum albumin was administered intravenously to 46 subjects in 53 experiments. Plasma, urine, fecal and ascitic fluid concentrations of radioactivity were followed for periods up to six weeks. Various body sites were assayed by *in vivo* techniques.

2. The labeled albumin reached distribution equilibrium in 4 to 7 days except in subjects with massive ascites where this period was prolonged. The apparent space of distribution in non-ascitic

subjects averaged about 2.5 times the plasma volume. Two apparent rates of transfer between plasma and extravascular spaces, with half times of approximately 3 and 24 hours, were observed.

3. Radioactivity released by degradation of the protein-iodine bond was almost completely accounted for by renal excretion in the presence of thyroid blockage.

4. Where observation periods were adequate, independent determinations of degradation rates from plasma and urine data agreed well. Most lots of I^{131} labeled albumin utilized contained variable quantities of rapidly degraded components. The major components in the best lots were degraded at a rate of about 4 per cent per day ($T_{1/2}$ = 17 days), or less, which agrees well with values obtained by biosynthetic methods but not with those previously reported by this method.

5. It is concluded that the validity of the I^{131} labeled albumin as a tracer for the study of endogenous albumin "turnover" rates has not been unequivocally demonstrated. Reasons for the disagreement with results of previous studies along the same lines are discussed.

APPENDIX A

Effect of renal removal of iodide on the renal excretion curve

The I^{131} of fragments released from the degraded protein is probably very rapidly converted to iodide, as has been demonstrated for di-iodotyrosine (14). At normal renal clearance rates, the half time for renal excretion of iodide is about nine hours (21), approximately 84 per cent being excreted in a 24 hour period.

Let

A = quantity of I^{131} tagged albumin in the body,
I = quantity of radioactive iodide in the body,
U = cumulative quantity of radioactive iodide in the urine,
λ_d = rate of degradation of iodo-albumin and release of I^{131} from the protein bond,
λ_r = rate of renal excretion of radioactive iodide,
then

$\frac{dU}{dt}$ = excretion of radioactive iodide per unit time.

Neglecting the very short time for conversion of radioactive iodinated tyrosine to radioactive iodide,

$$\frac{dA}{dt} = -\lambda_d A \qquad (1)$$

$$\frac{dI}{dt} = -\lambda_r I + \lambda_d A \qquad (2)$$

$$\frac{dU}{dt} = \lambda_r I. \qquad (3)$$

Integrating and substituting, we obtain:

$$A = A_0 e^{-\lambda_d t} \qquad (4)$$

$$I = \frac{\lambda_d A_0}{\lambda_r - \lambda_d} (e^{-\lambda_d t} - e^{-\lambda_r t}) \qquad (5)$$

$$\frac{dU}{dT} = \frac{\lambda_r \lambda_d A_0}{\lambda_r - \lambda_d} (e^{-\lambda_d t} - e^{-\lambda_r t}) \text{ (renal excretion curve).} \qquad (6)$$

If all the radioiodine released by iodo-albumin degradation were instantly excreted in the urine:

$$\frac{dU}{dt} = \lambda_d A \qquad (7)$$

substituting (4)

$$\frac{dU}{dt} = \lambda_d A_0 e^{-\lambda_d t} \text{ (albumin degradation curve).} \qquad (8)$$

Since λ_r is of the order of 25 to 40 times as great as λ_d even at moderately low renal clearance rates, it is clear, that within a short time, the second term in the brackets of equation (6) becomes relatively insignificant and the curves of (6) and (8) become virtually identical except for slightly different intercepts. Theoretical curves have been plotted for equations (6) and (8) with half times for renal removal of iodide and albumin degradation of 9 hours (0.375 days) and 15 days, respectively. It is apparent (Figure 11) that, at these rates, the renal excretion curve becomes virtually identical with the curve of iodo-albumin degradation after the first day or so.

APPENDIX B

Effect of multiple components on the characteristics of the plasma concentration and the renal excretion curves

Let P and Q be quantities of different iodo-albumins, p and q, degraded at rates λ_p and λ_q, respectively, where $\lambda_p > \lambda_q$. Then,

$$P = P_0 e^{-\lambda_p t}$$
$$Q = Q_0 e^{-\lambda_q t}.$$

If the apparent space of distribution remains constant, the total amount of iodo-albumin, A, at any time, t, remains constantly proportional to the plasma concentration of iodo-albumin, c. Thus A = kc where k is a constant equal to the apparent space of distribution. Disregard the mixing phase during which the iodo-albumin reaches equal specific activity in all compartments of the albumin pool. Then,

$$A = kc = P_0 e^{-\lambda_p t} + Q_0 e^{-\lambda_p t}. \qquad (1)$$

Assume that all degraded protein radioactivity appears in the urine immediately, which is a good approximation (see Appendix A). Then,

$$u = -\frac{dA}{dt} = \lambda_p P_0 e^{-\lambda_p t} + \lambda_q Q_0 e^{-\lambda_q t} \qquad (2)$$

u = renal excretion per unit time.

Equation (1) is essentially the plasma concentration curve and equation (2) is the urine excretion curve. Both obviously have the same form being resolved into two exponential terms. The corresponding slopes are identical but have different relative intercepts.

Since $\lambda_p > \lambda_q$,

$$\frac{\lambda_p P_0}{\lambda_q Q_0} > \frac{P_0}{Q_0}.$$

The renal excretion curve, therefore, has a relatively larger first term than the plasma concentration curve. It follows, then, that the renal excretion curve will have a sharper slope at all times than the plasma concentration curve, that this difference will be more marked in the earlier segments and that the approach to a single exponential straight line decay of the terminal segment will be apparent earlier in the plasma concentration curve. These are the very characteristics of the curves observed in these studies.

APPENDIX C

Exchange of iodo-albumin between plasma and ascitic fluid following intravenous administration

Constancy of albumin concentrations, volumes of the various compartments and transfer rates are assumed. Since exchange between plasma and ascitic fluid is rapid compared with the rate of degradation, differences in specific activity in the two compartments resulting from differential degradation in the compartments is neglected.

Let

P' = total iodo-albumin in plasma compartment, p, containing P grams albumin

F' = total iodo-albumin in ascitic fluid compartment, f, containing F grams albumin

Q' = total iodo-albumin in all other extravascular compartments, q, containing Q grams albumin

$P' + Q' = M'$ = total extraperitoneal iodo-albumin.

a) Under the assumption that equilibrium between p and q is rapid as compared with that between p and f, m may be regarded as a single pool in exchange with f. Then,

$$\frac{dM'}{dt} = -\lambda_m M' + \lambda_f F' \qquad (1)$$

$$\frac{dF'}{dt} = -\lambda_f F' + \lambda_m M' \qquad (2)$$

where

λ_m is the rate of transfer of iodo-albumin from m to f

λ_f is the rate of transfer of iodo-albumin from f to m

and at equilibrium

$$\lambda_m M' = \lambda_f F'.$$

Solving equations (*1*) and (*2*) and integrating, we obtain,

$$F' = (M'_0 - M'_E)(1 - e^{-(\lambda_f + \lambda_m)t}) \qquad (3)$$

$$M' = M'_E + (M'_0 - M'_E)e^{-(\lambda_f + \lambda_m)t} \qquad (4)$$

where M'_0 and M'_E are zero-time and equilibrium values, respectively.

In subject H, the ascitic fluid volume was not measured during the period of equilibration. Total ascitic fluid iodo-albumin was estimated as the difference between total exchangeable albumin and 2.5 times total plasma albumin (since the ratio of extravascular to plasma albumin in nonascitic subjects is approximately 60/40). The values obtained for endogenous albumin in the various compartments are then

TEA = 354 grams (Total exchangeable albumin)
P = 110 grams
Q = 165 grams
F = 79 grams.

The ascitic fluid albumin concentration was 1.18 grams per 100 ml. indicating an ascitic fluid volume of 7.5 liters which appeared reasonable.

Since

$$\lambda_m M = \lambda_f F.$$

Then,

$$\frac{\lambda_m}{\lambda_f} = \frac{F}{M} = \frac{79}{275}. \qquad (5)$$

From the ascitic fluid distribution curve (Figure 6) and, equation (*3*)

$$\lambda_m + \lambda_f = .0150 \text{ per hour.} \qquad (6)$$

Solving (*5*) and (*6*) we obtain,

λ_f = .0117 per hour
λ_m = .0033 per hour.

The rate of transfer of albumin across the peritoneal surface in either direction is then

$$\lambda_f F = \lambda_m M = .92 \text{ gram per hour.}$$

b) Under the assumption that plasma albumin is distributing into ascitic fluid alone, the resulting equations derived similarly to (*3*) and (*4*) are

$$F' = (P'_0 - P'_E)(1 - e^{-(\lambda_f + \lambda_p)t}) \qquad (7)$$

$$P' = P'_E + (P'_0 - P'_E)e^{-(\lambda_f + \lambda_p)t} \qquad (8)$$

where P'_0 and P'_E are zero time and equilibrium values, respectively, λ_p is the transfer rate of iodo-albumin from plasma to ascitic fluid and λ_f is the transfer rate of iodo-albumin from ascitic fluid to plasma. Now, if the same values for P and F are taken as in Method a) and solved in the same manner,

λ_f = 0.0087 per hour
λ_p = 0.0063 per hour
$\lambda_f F = \lambda_p P$ = .69 gram per hour.

The true value probably lies between the values obtained by Method a) and Method b). During the period of study there was a net increase in ascitic fluid albumin of approximately 4 grams per day (see text). Thus, the rate for passage from plasma to ascitic fluid was 0.17 gram per hour greater than in the reverse direction.

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